Recommendations for Clinical CYP2C19 Genotyping Allele Selection: A Report of the Association for Molecular Pathology

Short running title: *CYP2C19* Allele Testing Recommendations

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ABSTRACT

This document was developed by the Pharmacogenetics (PGx) Working Group of the Association for Molecular Pathology (AMP) Clinical Practice Committee, whose aim is to recommend variants for inclusion in clinical pharmacogenetic testing panels. The goals of the AMP PGx Working Group are to define the key attributes of PGx alleles recommended for clinical testing, and to define a minimum set of variants that should be included in clinical PGx genotyping assays. These recommendations include a minimum panel of variant alleles (Tier 1) and an extended panel of variant alleles (Tier 2) that will aid clinical laboratories when designing PGx assays. The Working Group considered variant allele frequencies in different populations and ethnicities, the availability of reference materials, as well as other technical considerations for PGx testing when developing these recommendations. These *CYP2C19* genotyping recommendations are the first of a series of recommendations for PGx testing. These recommendations are not to be interpreted as restrictive but to provide a helpful guide.

Introduction

This document describes recommendations from the Association for Molecular Pathology (AMP) Pharmacogenomics (PGx) Working Group for a minimum set of alleles to include in clinical cytochrome P450 2C19 (*CYP2C19*) genotyping panels. These recommendations are intended to inform clinical laboratory professionals when designing and validating clinical pharmacogenetic (PGx) assays, to promote standardization of PGx testing across different laboratories, and to complement other clinical guidelines such as those issued by the Clinical Pharmacogenetics Implementation Consortium (CPIC), which primarily focuses on the interpretation of genotyping results and therapeutic recommendations for specific drug(s).[1] The specific aims of this Working Group are to define the key attributes of alleles that are recommended for clinical PGx testing and to recommend variants for inclusion in clinical *CYP2C19* testing panels.

Reasons for Pharmacogenetic Test Standardization

In the United States, clinical laboratories performing genetic testing must comply with the Clinical Laboratory Improvement Amendment (CLIA) standards and guidelines for clinical genetic testing (Electronic Code of Federal Regulations Part 493—Laboratory Requirements: Clinical Laboratory Improvement Amendments of 1988, https://www.ecfr.gov/cgi-bin/text-idx?SID=1248e3189da5e5f936e55315402bc38b&node=pt42.5.493&rgn=div5, last accessed 8/25/2017). These regulations require clinical laboratories to include the list of interrogated PGx variants and star (*) alleles, and assay limitations in their reports; however, there currently are no professional recommendations on which variants to include in clinical PGx tests. A recent Genetic Testing Reference Material Program (GeT-RM) study utilized a number of PGx test panels, and examination of the assay designs revealed that the variants included were not consistent between panels.[2] Without exception, no two tests that examined any of the 28 PGx genes included in the study were designed to detect the same set of variants and/or haplotypes (alleles).[2] Similar findings were recently reported by Moyer et al

when they surveyed laboratories offering PGx services for *CYP2D6* and *CYP2C19* genotyping.[3] In addition, some tests used different combinations of variants to define haplotypes and results were reported using different nomenclature systems (eg, Human Genome Variant Society,[4] dbSNP,[5] star (*) alleles).[6] Variations in test design and nomenclature can impact haplotype and diplotype assignment, and ultimately alter test interpretation and patient care. Notably, the AMP has previously supported CPIC's efforts in standardizing PGx allele function and phenotype nomenclature (https://www.amp.org/AMP/assets/File/position-statements/2015/AMPendorsementoftheCPICinitiative2015-10-26.pdf, last accessed 6/23/2017).

Defining the key attributes of PGx alleles recommended for clinical testing, along with a minimum set of variants that should be included in clinical PGx genotyping test panels, as a strategy is analogous to the 2001 and 2004 recommendations developed by the American College of Medical Genetics and Genomics (ACMG)/American College of Obstetricians and Gynecologists (ACOG) of a panel of 25 *CFTR* pathogenic variants (later revised to 23) for cystic fibrosis carrier (CF) screening in the US[7,8] (ACMG, https://www.acmg.net/Pages/ACMG_Activities/stds-2002/cf.htm , accessed 1/30/18). Although many clinical laboratories often include other *CFTR* pathogenic variants in addition to the 23 recommended pathogenic variants (National Center for Biotechnology Information, https://www.ncbi.nlm.nih.gov/gtr/conditions/C0010674/, accessed 1/30/18), adoption of these ACMG/ACOG recommendations helped to standardize CF carrier testing in the US and make the development and evaluation of assays more transparent.

Considering the complex nature of clinical PGx testing and interpretation, and its impact on patient care, there is a practical need for standardizing clinical PGx assays. Standardized test panels developed for clinical PGx assays could also facilitate clinical PGx implementation. Variants and alleles for each gene could be selected using criteria such as population frequency, level of supporting evidence for phenotypic outcome, clinical utility, and severity of adverse drug reaction or loss of efficacy. Such

standardized PGx test panels could enable physicians, pharmacists, researchers, and other stakeholders to understand PGx test results without extensive scrutiny of the alleles included in the assay, and provide assurance that clinical laboratory panels include a minimum set of clinically relevant PGx variant alleles. This is especially important when test results, originally obtained to assist selection and dosing of one medication, are used later in the patient's life for selection and dosing of a different medication. As with *CFTR* testing, laboratories could add additional variants to their panels for expanded clinical PGx testing; however, all commercial PGx assays and laboratory-developed tests would theoretically contain a consistent minimum set of variants. Evidence-based recommendations developed by professional organizations often form the basis for future laboratory accreditation requirements.

The AMP PGx Working Group cited important issues that are unique to pharmacogenetics which need to be considered when developing standardized panels. First, allele function derived from *in vitro* models may not directly translate to a clinical phenotype or metabolizer status (ie, poor metabolizer, intermediate metabolizer, normal metabolizer, rapid metabolizer, and ultrarapid metabolizer), and allele function can be substrate and/or drug concentration dependent. Although an allele may be extremely rare in the general population, it may be more frequent in a specific ethnic population or in a phenotypically selected group of patients (eg, those with side effects on specific drugs). Therefore, the inclusion of rare, but potentially clinically significant alleles in recommended panels needs to be considered. Second, like other PGx genes, variants in *CYP2C19* are typically reported as star (*) allele inferred haplotypes and diplotypes. A haplotype refers to a set of DNA sequence variants including single nucleotide variants (SNVs) or structural variants inherited as a unit on a single chromosome. The combination of haplotypes from both chromosomes is generally reported together as a diplotype. The diplotype is usually translated into a predicted phenotype or metabolizer status which clinicians use for prescribing drugs. For *CYP2C19*, alleles negative for the assayed sequence variants are designated as *1, which is typically the most common or normal function allele. However, the *1 designation may not be

accurate if additional, undetected variants are present. The known *CYP2C19* star (*) alleles are cataloged by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee (https://www.pharmvar.org/gene/CYP2C19, last accessed 11/30/2017). *CYP2C19* alleles definitions are also available through PharmGKB (https://www.pharmgkb.org/gene/PA124#tabview=tab4&subtab=31, last accessed 6/20/2017). The list of recommended alleles will require periodic re-evaluation as novel variants are identified and characterized, and new insights into genotype-phenotype relationships are established. Each new allele will need to be evaluated by the same criteria to assess importance and added to the standardized test panel if the criteria are met. In some cases, further research may determine that an allele previously on the recommended test panel no longer meets the required criteria.

The availability of reference materials (RMs) was an important consideration for recommendation of inclusion of *CYP2C19* alleles in clinical genotyping test panels. Well-characterized RMs are fundamental to laboratory quality assurance (QA) programs, internal QA activities such as quality control (QC), test development/validation,[9] and periodic external assessment by proficiency testing (PT). Selection of appropriate RMs is based on the needs of the assay, test methodology, and availability. To meet the need for publicly available, characterized RMs, the Centers for Disease Control and Prevention (CDC), in partnership with the clinical testing community, established the GeT-RM Program (http://wwwn.cdc.gov/clia/Resources/GetRM/ , last accessed 6/20/2017). Its goal is to improve the supply of publicly available and well-characterized genomic DNA that can be used as RM for PT, QC, test development/validation, and research studies. In conjunction with AMP, GeT-RM coordinated the characterization of 107 DNA samples for clinically relevant polymorphisms for PGx loci, including selected *CYP2C19* alleles.[10] A second study characterized 137 selected DNA samples for 28 PGx genes, including *CYP2C19*.[2] These DNA samples are publicly available through the Coriell Cell Repositories (Camden, NJ).

Although high-throughput DNA sequencing has become more common, many PGx genes are difficult to interrogate by commonly used capture-based short-read sequencing platforms due to sequence homologies and interference of pseudogenes.[11] Regardless of the methodology, the AMP PGx Working Group believes that establishing recommendations for a minimum set of alleles/variants that should be included in clinical PGx test panels is an important step forward to ensure better standardization and inter-laboratory concordance of PGx testing. This AMP PGx document on *CYP2C19* allele selection is intended to be the first of a series of PGx gene recommendations for laboratories offering clinical PGx testing.

Goals of Recommendations

As noted above, the goals of the AMP PGx Working Group are to define the key attributes of PGx alleles recommended for clinical testing, and to define a minimum set of variants that should be included in clinical PGx genotyping assays. These recommendations include a minimum panel of variant alleles (Tier 1) and an extended panel of variant alleles (Tier 2) that will aid clinical laboratories when designing PGx assays. When developing these recommendations, the Working Group considered variant allele frequencies in different populations and ethnicities, the availability of RMs, as well as other technical considerations for PGx testing. The aim is that these recommendations will facilitate more consistent PGx testing across clinical laboratories.

Gene: *CYP2C19*

CYP2C19, a member of the cytochrome P450 superfamily, is involved in the phase I metabolism of many commonly prescribed medications, including the antiplatelet agent clopidogrel, proton-pump inhibitors, and the selective serotonin reuptake inhibitors (SSRIs) antidepressants citalopram/escitalopram.[12,13] The *CYP2C19* gene is located on chromosome 10q23.33 and is highly polymorphic, which can lead to large inter-individual variation in CYP2C19 enzyme activity and related drug response phenotypes and/or undesired adverse drug events.[14] *CYP2C19* is one of the most

frequently tested PGx genes in clinical laboratories and is included in the Food and Drug Administration (FDA) Table of Pharmacogenetic Biomarkers in Drug Labeling for several FDA-approved drugs (https://www.fda.gov/Drugs/ScienceResearch/ucm572698.htm, last accessed 1/30/2018).

According to The Genetic Testing Registry (https://www.ncbi.nlm.nih.gov/gtr/all/?term=CYP2C19, last accessed 2/12/2017), the *CYP2C19* variants tested in US clinical laboratories range from a few targeted alleles to the entire coding region. The genetic tests utilize a variety of methods including targeted variant detection, bidirectional Sanger DNA sequencing, and next-generation sequencing. Factors that lead to inconsistent clinical *CYP2C19* testing between laboratories includes the choice of tested alleles, targeted testing of populations with varying ethnic backgrounds, as well as technical performance of the various platforms. For example, laboratories that service a particular ethnic population based on geography may test only for variants that are specific to that particular population.

Existing Guidelines

Clinical PGx practice guidelines are available from additional professional organizations, including CPIC (https://cpicpgx.org/guidelines/, last accessed 1/30/2018), the Dutch Pharmacogenetics Working Group (DPWG)[15], and the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) (http://cpnds.ubc.ca/about/projects, last accessed 6/20/2017). These guidelines, which were developed using an extensive literature review and discussion among consortium of individual volunteers who have experience or have published on the specific drug or gene, are gene-drug pair oriented with an emphasis on interpretation of genotype and phenotype, and genotype-guided therapeutic recommendations. These drug-oriented guidelines have played a critical role in facilitating the clinical implementation of PGx and have enabled the development of clinical decision support tools for clinicians to understand and more efficiently utilize PGx testing results. There are currently 21 dosing recommendations available

on CYP2C19-metabolized medications from the CPIC and DPWG (PharmGKB,[16] https://www.pharmgkb.org/gene/PA124, last accessed 6/20/2017). Although all guidelines include lists of common *CYP2C19* alleles reported to date in various populations and their functional and clinical relevance, they do not explicitly recommend specific variant alleles for clinical laboratories to include in their *CYP2C19* testing platforms. Despite the fact that many commercial platforms are available, clinical laboratories often develop their own assays for *CYP2C19* testing. Specific considerations from a clinical laboratory perspective such as *CYP2C19* allelic variant selection, testing platforms, and RMs are not addressed in the CPIC or DPWG practice guidelines.

CYP2C19 Alleles Recommendation

The AMP PGx Working Group reviewed the variant *CYP2C19* star (*) alleles currently cataloged by the CYP450 Allele Nomenclature Committee, including allele function status, multiethnic allele frequencies, the availability of RMs, and commercially available genotyping platforms (Table 1). Tier 1 *CYP2C19* variant alleles were defined as those that have: i) well-characterized alteration of CYP2C19 activity that has been shown to have an effect on drug response and for which the functional variant is known, ii) appreciable minor allele frequency in a patient population, and iii) available RMs (Table 2).[2] Tier 2 variant *CYP2C19* alleles are defined as alleles that meet at least one but not all of the criteria for inclusion in Tier 1, and are considered optional for inclusion in expanded clinical genotyping panels. These include normal function variant alleles, low frequency alleles, and alleles without currently available RMs. Some of the Tier 2 alleles may become Tier 1 in the future if RMs or additional information becomes available. Variants with unknown or uncertain function are not recommended for inclusion in targeted clinical *CYP2C19* genotyping test panels, although it may be useful to include these in research panels to clarify functional and clinical outcomes.

Tier 1 *CYP2C19* Variant Alleles

CYP2C19 variant alleles considered as Tier 1 by the AMP PGx Working Group include **2*, **3,* and **17*. This was based on their well-established functional effect on CYP2C19 activity and drug response,[17] availability of RMs, and their frequencies in major ethnic groups. The major no function *CYP2C19*2* allele is characterized by the presence of a synonymous transition in exon 5 that results in abnormal splicing (NM_000769.2:c.681G>A; p.Pro227Pro; rs4244285).[18] *CYP2C19*2* has a minor allele frequency ranging from 13% to 54% in most major racial and ethnic groups, and consequently is the most common *CYP2C19* allele interrogated by clinical laboratories. The no function *CYP2C19*3* allele is a nonsynonymous transition in exon 4 that results in a premature stop codon (NM_000769.2:c.636G>A; p.Trp212Ter; rs4986893).[19] Although it has a low minor allele frequency (<1%) in most major racial and ethnic groups, it has a frequency of ~8% to 10% in the East Asian population. The increased function *CYP2C19*17* allele is characterized by a promoter variant that results in increased gene expression (NM_000769.2:c.-806C>T; rs12248560).[20] Importantly, the inclusion of these three variants accounts for 43% to 100% of the currently defined variant *CYP2C19* alleles in most major racial and ethnic groups (Table 3).[17,21] In terms of the clinical impact of the recommended Tier 1 recommended alleles, patients with *CYP2C19 *1/*17* and **17/*17* (rapid and ultra-rapid metabolizer) diplotypes are more likely to have lower than desired therapeutic levels of some medications such as voriconazole,[17] sertraline,[22] and citalopram,[22] whereas patients with *CYP2C19 *2/*2*, **2/*3*, or**3/*3* (poor metabolizer) diplotypes are more likely to have higher than normal levels of these medications with an increased risk for adverse events. Conversely, for the anti-platelet therapy clopidogrel, a prodrug which requires bioactivation by CYP2C19, ultra-rapid metabolizers have higher plasma concentrations of the active metabolite, whereas poor metabolizers have reduced plasma concentrations of the active metabolite and reduced efficacy of anti-platelet inhibition.[23]

Tier 2 *CYP2C19* Variant Alleles

The following *CYP2C19* alleles were recommended as Tier 2: **4A*, **4B*, **5*, **6*, **7*, **8*, **9*, **10*, **35* (Table 4).[17] These alleles have been shown to have decreased or no function (https://www.pharmgkb.org/page/cyp2c19RefMaterials, last accessed 1/30/2018). Moreover, the Tier 2 alleles have a combined frequency higher than 1% in several major ethnic groups, with the highest combined frequency (13%) being observed in populations of African descent[17]. The most common Tier 2 allele is the **35* allele (NM_000769.2:c.332-23A>G; rs12769205) which is found at 9% frequency in Africans. Although these alleles are included in many platforms, they were not included in the Tier 1 recommendations due to either low minor allele frequency (which can result in an increase of false positive results), less well-characterized impact on CYP2C19 function, or a lack of RMs. Specifically, the **4A*, **4B*, **5*, **6*, **7*, and **8* alleles have frequencies less than 0.5% in major ethnic groups; **9* and **10* are decreased function alleles and, therefore, their clinical significance is uncertain (https://www.pharmgkb.org/page/cyp2c19RefMaterials, last accessed 7/26/2017); and RMs are not available for **5*, **7*, **35*. Given that **35* was only recently characterized[21], it was not interrogated in the recent CDC GeT-RM study and thus there is no known RM available; nor is it included among the majority of currently available commercial platforms (Table 1). As further information is gained about *35 and RM becomes available, it may be promoted to a Tier 1 allele.

Of note, the defining variant of the no function *CYP2C19*4* allele (NM_000769.2:c.1A>G; rs28399504) can be in linkage equilibrium with the defining variant of *CYP2C19*17* (NM_000769.2:c.-806C>T; rs12248560) in certain ethnic subpopulations and this haplotype has been designated *CYP2C19*4B*. Therefore, to distinguish between the no-function *CYP2C19*4B* and the increased function *CYP2C19*17*, both defining variants (rs28399504 and rs12248560) need to be interrogated, and preferably by a method that can determine their phase.[24,25] Additionally, a rare nonsynonymous *CYP2C19* variant (NM_000769.2:c.463G>T; p.Glu155Ter; rs374036992) also can occur in linkage

disequilibrium with the defining variant of *CYP2C19*17* (NM_000769.2:c.-806C>T; rs12248560) and this haplotype has yet to be assigned an independent star (*) allele designation by the CYP Allele Nomenclature Committee. Although it could be useful to interrogate this rare variant and distinguish *CYP2C19*17* from this novel haplotype, the absence of an independent star (*) allele designation, its low frequency, lack of available RM, and its unclear clinical importance limits its inclusion in clinical *CYP2C19* testing panels.[26]

DISCUSSION

It is important for molecular diagnostic laboratories to have resources to guide them in their efforts to offer adequate PGx testing. AMP believes that it is the responsibility of professional organizations to establish guidelines for professional practice. Our members are among the early adopters and users of PGx testing in clinical settings, and have accumulated substantial knowledge and expertise as it relates to this area of clinical testing. This document offers a two-tier categorization of *CYP2C19* alleles as an aid for designing *CYP2C19* genotyping assays.

Using criteria such as allele function, population frequency, and availability of RMs, the AMP PGx Working Group has proposed a recommended minimum set of alleles and their defining variants (Tier 1) that should be included in clinical *CYP2C19* PGx tests. In addition, we have defined a list of *CYP2C19* alleles that do not currently meet one or more of the criteria for inclusion in Tier 1 and are thus considered optional for clinical testing (Tier 2). These recommendations are intended to facilitate testing by laboratories and to improve genotyping concordance across laboratories.

There are currently four CPIC guidelines for CYP2C19 covering 10 medications: SSRIs;[22] tricyclic antidepressants (TCAs);27 clopidogrel;[23] and voriconazole.[17] One guideline issued by the DPWG offers recommendations for four additional proton pump inhibitors.[15] Moreover, within the FDA prescribing label, actionable CYP2C19 genotype information is available to physicians for five additional drugs:

carisoprodol (Soma Prescribing Label 02/01/2013, www.accessdata.fda.gov/drugsatfda_docs/label/2013/011792s045lbl.pdf; last accessed 07/11/ 2017), clobazam (Onfi Prescribing Label 12/16/2016, www.accessdata.fda.gov/drugsatfda_docs/label/2016/202067s004lbl.pdf; last accessed 07/11/ 2017), diazepam (Diastat Prescribing Label 12/16/2016, www.accessdata.fda.gov/drugsatfda_docs/label/2016/020648s014lbl.pdf; last accessed 07/11/ 2017) brivaracetam (Briviact Prescribing Label 06/03/2016, www.accessdata.fda.gov/drugsatfda_docs/label/2016/205836s001,205837s001,205838s001lbl.pdf; last accessed 07/11/ 2017), flibanserin (Addyi Prescribing Label 08/18/2015, www.accessdata.fda.gov/drugsatfda_docs/label/2015/022526lbl.pdf; last accessed 07/11/ 2017). Consequently, CYP2C19 PGx testing has the potential to guide clinicians when considering the use of at least 15 different medications some of which are amongst the top 200 most prescribed medications in the US (http://clincalc.com/DrugStats/, last accessed 11/30/2017).

Tier 2 *CYP2C19* alleles are additional variant alleles that could be included in clinical assays. These alleles either have low minor allele frequencies, lack available RMs, or have limited documented effects on CYP2C19 function. This represents an opportunity for laboratories to refine their PGX test and expand its coverage by including additional Tier 2 alleles. For example, given the frequency of the no function *CYP2C19*35* allele in African populations, inclusion of this allele may more accurately assign a diplotype and a subsequent phenotype for this ethnic group.

These recommendations for *CYP2C19* genotyping do not evaluate the evidence for clinical significance or utility, nor do they include information on *CYP2C19* genotype interpretation or reporting, as these were considered to be out of scope for this document and/or available from other sources (eg, PharmGKB, CPIC). However, clinical laboratories that offer *CYP2C19* genotyping should be aware that different *CYP2C19* variant alleles may have distinct clinical implications based on their functional status

and can be medication-specific. For example, the no function *CYP2C19* alleles (eg, **2* and **3*), which can result in the *CYP2C19* poor metabolizer phenotype, are more relevant to clopidogrel responsiveness testing,[23] whereas the increased function *CYP2C19*17* allele, which can result in the *CYP2C19* rapid and ultra-rapid metabolizer phenotypes, is more relevant to dosing voriconazole[17] and the SSRIs.[22]

Pharmacogenetics is a rapidly changing field; therefore, this document is limited to an evaluation of the current knowledge base. The AMP PGx Working Group intends to update this recommendation document as new evidence and/or RMs become available. The evidence available in the existing literature also informed the recommended list of 16 Tier 2 alleles. We anticipate that some of these alleles could be updated to Tier 1 alleles as RMs become available and/or as new frequency information for specific ethnicities is identified. We recognize that other known *CYP2C19* alleles are not listed in this recommendation as there are over 35 *CYP2C19* alleles listed in the CYP allele nomenclature database [https://www.pharmvar.org/gene/CYP2C19, last accessed 1/30/2018)]. Some of these could be updated to Tier 2 or even Tier 1 alleles based on new data concerning function, clinical relevance, frequency, and future availability of RMs.

Implementation of this recommendation document is at the discretion of the laboratory. The Tier 1 alleles are currently included in the available PT programs (eg, College of American Pathologists North American Specialized Coagulation Laboratory Association). The majority of laboratories participating in these PT programs currently test for all of the Tier 1 alleles.[28] This indicates that many clinical laboratories are already testing the Tier 1 alleles and that these recommendations would be practical to implement and reinforce standardization between laboratories.

This document is limited to recommendations of alleles to include in clinical laboratory assays and does not include information about *CYP2C19* genotype-to-phenotype relationship or clinical interpretation for *CYP2C19* results such as changes to medication therapy. Correlation of genotypes to *CYP2C19* phenotype expressed as a metabolizer status and recommendations for clinical actions based

on *CYP2C19* genotypes are included in the clinical practice guidelines published by CPIC, DPWG, and other professional societies and regulatory bodies.

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Table 1. Commercial assays currently available for *CYP2C19* genotyping.

Platform	*2	*2A	*2B	*3	*4	*4A	*4B	*5	*5A	*5B	*6	*7	*8	*9	*10	*12	*13	*14	*15	*17	*35	Regulatory status†
Affymetrix Pharmacoscan		X	X	X	X			X			X	X	X	X	X	X	X	X	X	X		RUO
Agena Biosciences (Sequenom) iPLEX ADME	X			X	X				X	X	X	X	X	X		X				X		RUO
Autogenomics INFINITI	X			X																X		FDA-cleared
GenMark eSensor	X			X	X			X			X	X	X	X	X		X			X		RUO
Luminex xTAG v3	X			X																X		FDA-cleared
LifeTech Taqman Open Array	V																					LDP
Spartan Bioscience	X			X																X		FDA-cleared

Not a comprehensive list. Inclusion herein does not represent an endorsement of any product or service by AMP.

†Regulatory status of commercially available platforms as of 8/31/17.

FDA, Food and Drug Administration; LDP, laboratory developed procedure; RUO, research use only; X, included; V, variable.

Table 2. Currently available Reference Materials for *CYP2C19.*

*allele	Coriell#
*2	NA17641(*2/*17)
	NA17673(*1/*2)
	NA20509 (*2/*2)
	NA18484 (*1 (*27)/*2)
	NA18564 (*2/*3)
	NA24009 (*2/*9)
	NA23874 (*2/*6)
	NA07439 (*2/*10)
	NA19122 (*1 (*15)/*2)
*3	NA18564 (*2/*3)
	NA23246 (*3/*17)
*4	NA18552 (*1/*4)
*4B	NA23878 (*1/*4B)
*5	none
*6	NA19178 (*1 (*27)/*6)
	NA23874 (*2/*6)
*7	none
*8	NA07029 (*8/*17)
	NA238739 (*1/*8)
*9	NA24009 (*2/*9)
	NA24008 (*9/*17)
*10	NA07439 (*2/*10)
*12	NA17074 (*1 (*12)/*17)
*13	NA19239 (*13/*17)
	NA17448 (*1/*13)
*15	NA19143 (*1/*15)
	NA19122 (*1 (*15)/*2)

*17	NA17641(*2/*17),
	NA12236(*1/*17),
	NA17074 (*1 (*12)/*17)
	NA19109 (*17/*17)
	NA19239 (*13/*17)
	NA07029 (*8/*17)
	NA24008 (*9/*17)
	NA23246 (*3/*17)
*27	NA19178 (*1 (*27)/*6)
	NA18484 (*1 (*27)/*2)
*35	none

This is not a comprehensive list. Inclusion herein does not represent an endorsement of any product or service by AMP. For a complete list, see CDC website. (https://wwwn.cdc.gov/clia/Resources/GETRM/default.aspx, last accessed 6/20/2017)).[2]

Table 3. *CYP2C19* Tier 1 variant alleles.

Allele	Allele Functional Status†	Defining Functional Variant	HGVS Nomenclature: NM_000769.2	HGVS Nomenclature: NG_008384.2‡	Reference Material Available	Multiethnic Allele Frequency
*2§	No function	rs4244285	c.681G>A	g.24154G>A	Yes	12-54%
*3	No function	rs4986893	c.636G>A	g.22948G>A	Yes	0.3-15%
*17	Increased function	rs12248560	c.-806C>T	g.4195C>T	Yes	4-21%

† Citations for assignment of function can be found at https://www.pharmgkb.org/page/cyp2c19RefMaterials, , last accessed 6/20/2017.

‡ CYP2C19 RefSeqGene.

§Note that the defining *2 variant (rs4244285) is most likely linked with the defining variant of the *35 allele (rs12769205); however, the *35 definition includes rs12769205 without rs4244285.[21]

Table 4. ***CYP2C19*** **Tier 2 variant alleles.**

Allele	Allele Functional Status	Defining Variant(s)	HGVS Nomenclature: NM_000769.2	HGVS Nomenclature: NG_008384.2†	Reference Material Available	Multiethnic Allele Frequency‡
*4A	No function	rs28399504	c.1A>G	g.5001A>G	Yes	0.1-0.3%
*4B	No function	rs28399504; rs12248560	c.[-806C>T; 1A>G]	g.[4195C>T;5001A>G]	Yes	0-0.2%
*5	No function	rs56337013	c.1297C>T	g.95033C>T	No	0%
*6	No function	rs72552267	c.395G>A	g.17748G>A	Yes	0-0.1%
*7	No function	rs72558186	c.819+2T>A	g.24294T>A	No	0%
*8	No function	rs41291556	c.358T>C	g.17711T>C	Yes	0.1-0.3%
*9	Decreased function	rs17884712	c.431G>A	g.17784G>A	Yes	0.1-4.2%
*10	Decreased function	rs6413438	c.680C>T	g.24153C>T	Yes	0.1-6%
*35§	No function	rs12769205	c.332-23A>G	g.17662A>G	No	0.8-3.1%

†CYP2C19 RefSeqGene; forward relative to chromosome).

‡From https://www.pharmvar.org/gene/CYP2C19, last accessed 6/20/2017/.

§Note that the defining *2 variant (rs4244285) is most likely linked with the defining variant of the *35 allele (rs12769205); however, the *35 definition includes rs12769205 without rs4244285.[21]